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Kinetic of the gibberellic acid and bikaverin production in an airlift bioreactor

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ABSTRACT

A mathematical description of the principal kinetics involved in gibberellic acid and bikaverin production in an airlift bioreactor was attained using a non-structured model. Growth, dextrose and nitrogen consumption, and gibberellic acid and bikaverin production kinetics were considered for this purpose. Experimental data were obtained from submerged batch fermentation of the fungus *Gibberella fujikuroi* (CDBB H-984) and were fitted to three different sigmoidal models: 2- and 3-parameter Gompertz models and a logistic model. The most appropriate model was determined by means of an *F* test using growth kinetic fitting. The 2-parameter Gompertz model was found to be the most suitable and was used for both dextrose and nitrogen consumption, and gibberellic acid and bikaverin production fitting. A sensitivity analysis was performed to determine the effect of the parameters in the global model. The parameters of the 2-parameter Gompertz model were transformed in order to give them a biological meaning.

1. Introduction

Gibberellic acid and bikaverin are well known secondary metabolites of the fungus *Gibberella fujikuroi*. Gibberellic acid is a hormone present in higher plants that regulates different growth processes, with agricultural applications [18]. Bikaverin is a red pigment with anti-protozoal [2] and anti-tumour [10] activity. Acetyl-CoA is the common precursor of these metabolites. However, their biosynthesis is carried out in different sub-cellular compartments and used in independent substrate pools [7]. Bikaverin is synthesized via the polyketide route whereas gibberellic acid is synthesized through the isoprenoid pathway.

Previous works have modelled bikaverin production in a fluidised bed bioreactor [5], using sigmoidal functions to explain growth kinetics, and in a stirred tank bioreactor [16] using Monod-type functions to explain growth kinetics. Gibberellic acid production modelling in solid-state fermentation is also described [11].

Sigmoidal models that are not coupled with substrate consumption are easy to employ and predict the growth kinetic of *G. fujikuroi* under different culture conditions [5,6,8,15] and [13]. These types of models allow the growth kinetic to be integrated separately from the substrate and production kinetic. This enables the production kinetic to be integrated from the point at which the nitrogen is exhausted. The point at which the gibberellic acid or bikaverin starts to be biosynthesised can then be established. Monod-type models are coupled with substrate consumption. More specifically, they are coupled with limiting substrate consumption. Since nitrogen is the limiting substrate, whether the fermentation is for gibberellic acid or bikaverin production, the model will predict no more growth once the nitrogen is totally consumed by the fungus. Due to the storage of fat and carbohydrate in the fungus after nitrogen is exhausted, the biomass will continue increasing. Moreover, if the model predicts a satisfactory nitrogen exhaustion time, this will affect the global model. The model will be simplified because of the reduction of the number of adjustable parameters, and the fitting capacity affected.

Both sigmoidal or Monod-type models facilitate fermentation analysis and enable information to be obtained in a practical way. Furthermore, once these models are robust, they can be used to describe the production process under different fermentation conditions such as temperature, pH or aeration, among others. Eventually, the models enable us to understand, design and control the fermentation process of these metabolites.

The mathematical description formulated in the present work addresses the process of gibberellic acid and bikaverin production in an airlift bioreactor, considering growth, substrate consumption and metabolite production kinetics.

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2. Materials and methods

2.1. Micro-organism

G. fujikuroi (Sawada) strain CDBB H-984 maintained on potato glucose slants at 4 °C and sub-cultured every 2 months was used in the present work (Culture collection of the Department of Biotechnology and Bioengineering, CINVESTAV-IPN, Mexico).

2.2. Inoculum preparation

Fully developed mycelial material from a slant was removed by adding an isotonic solution (0.9% NaCl). The mycelium is used to inoculate 300 ml of fresh culture medium contained in an Erlenmeyer flask. The flask is placed in a gyratory shaker (200 rpm) for 38 h at 29 ± 1 °C. Developed mycelia will be used to inoculate the culture medium contained in the airlift bioreactor. The culture medium employed for the inoculum preparation is reported by Barrow et al. [3].

2.3. Batch culture in the airlift bioreactor

An Applikon airlift bioreactor Netherlands (working volume; 3.5 l) was employed in the present work. It consists of two concentric tubes with a settler where the air enters the bioreactor through the inner tube. The bioreactor is surrounded by a jacket filled with water allowing temperature control. It is also equipped with pH and dissolved oxygen sensors to control these variables. Moreover, it allows material from the bioreactor to be fed or removed by means of peristaltic pumps. Typical culture medium contained glucose (50 g l^{-1}), NH_4Cl (0.75 g l^{-1}), KH_2PO_4 (5 g l^{-1}), $MgSO_4{\cdot}7H_2O$ (1 g l^{-1}), trace elements (2 ml l^{-1}). Typical fermentation conditions were pH set to 3.0, temperature set to 29 °C and aeration rate set to 1.6 vvm. At pre-determined times a sub-sample was withdrawn from the bioreactor and was fully analysed.

2.4. Analytical methods

The dry weight method was employed to determine biomass concentration. Reducing sugars were determined by the DNS method [14]. Nitrogen concentration was determined by a modified Kjeldahl method [1]. Gibberellic acid concentration was determined with an HPLC method developed by Castillo and Martinez [4] and bikaverin concentration was determined by measuring absorbance at 520 nm.

2.5. Mathematical models

Three different sigmoidal models were considered for the kinetic modelling of gibberellic acid and bikaverin production: 2-parameter (Eq. (1)) and 3-parameter (Eq. (2)) Gompertz models and a logistic model (Eq. (3)). These models were not coupled with substrate consumption.

$$\frac{dx}{dt} = kx\,e^{-\mu t} - ax \tag{1}$$

$$\frac{dx}{dt} = kx\,e^{-\mu t} \tag{2}$$

$$\frac{dx}{dt} = kx - ax^2 \tag{3}$$

where x is the biomass concentration (g l^{-1}), k is a kinetic parameter related to initial specific growth rate (h^{-1}), a is a kinetic parameter related to growth inhibition, μ is the specific growth rate (h^{-1}) and t is the time (h) Eq. (1) contains three adjustable parameters while Eqs. (2) and (3) contain two adjustable parameters. Eqs. (1) and (2) consider a self-limited growth where the rate decreases exponentially with time. Eq. (1) has an extra term that takes into account inhibition, and has been used by Escamilla-Silva et al.[8] and Negrete [15] in gibberellic acid production modelling by *G. fujikuroi*. Eq. (3) describes exponential growth with an inhibition factor proportional to x^2. The Logistic model has been used by Machado et al. [13] in describing the growth kinetic of *G. fujikuroi* in solid-state fermentation.

Eq. (4) describes dextrose consumption and Eq. (5) describes nitrogen consumption. These last two equations consider parallel consumption of the substrate for growth and for maintenance requirements. Eq. (6) is the classic Leudeking–Piret model which combines growth- and non-growth associated contributions to product formation. Eq. (6) will be used to describe both gibberellic acid and bikaverin production kinetics.

$$\frac{ds}{dt} = -\frac{1}{Y_{x/s}}\frac{dx}{dt} - m_s x \tag{4}$$

$$\frac{dn}{dt} = -\frac{1}{Y_{x/n}}\frac{dx}{dt} - m_n x \tag{5}$$

where s is the dextrose concentration (g l^{-1}), $Y_{x/s}$ is the yield factor for dextrose, m_s is the maintenance coefficient for dextrose (h^{-1}), n is the nitrogen concentration (g l^{-1}), $Y_{x/n}$ is the yield factor for nitrogen and m_n is the maintenance coefficient for nitrogen (h^{-1}).

$$\frac{dp}{dt} = \alpha\frac{dx}{dt} + \beta x \tag{6}$$

where p is the product concentration (mg l^{-1}) and α and β are Leudeking–Piret constants.

For model fitting, Eqs. (1)–(3) were integrated via the Runge-Kutta technique and parameter values were optimised by means of GREG subroutine [17], minimizing the residual sum of squares (RSS) (as named in Microsoft Excel®) using a DLL created by Digital Visual Fortran®. Following model discrimination, the selected model was used in Eqs. (4)–(6) and the integration and optimisation process was repeated. 13 different data from fermentations of gibberellic acid production and 14 different data from fermentations of bikaverin production were analysed and parameter estimation was carried out by means of the minimization of residual sum of squares.

2.6. Model discrimination

Model discrimination was carried out by means of an F test under the assumption that the 3-parameter model exactly predicts the concentration of biomass at every moment, according to the method described by Zwietering et al. [19]. The following was calculated:

$$f = \frac{(RSS_2 - RSS_1)/(DF_2 - DF_1)}{RSS_1/DF_1} \tag{7}$$

where RSS_2 is the residual sum of squares value from the 2-parameter model, RSS_1 is the RSS value from 3-parameter Gompertz model, DF_1 is the degrees of freedom number from the 3-parameter Gompertz model and DF_2 is the degree of freedom number from the 2-parameter model. This analysis is an approximation due to comparison of non-linear models and was also used by Chavez-Parga et al. [5,6] for model discrimination.

3. Results and discussion

All data analysed in this work were reported by Chavez-Parga et al. [5,6]. For gibberellic acid production 13 different kinetic data were considered and for bikaverin production, fourteen. All experiments were performed under different conditions of nutrients and air-flow rate. Typical growth kinetics for gibberellic acid and bikaverin production are shown in Figs. 1 and 2, respectively. As can be seen, there is no lag phase. This is due to the similarity between the inoculum and fermentation medium. Therefore, the curve starts with a rapid growth phase where the dry weight increases exponentially. Once the limiting nutrient starts to decline, the dry weight stops increasing exponentially but continues to increase due to fat and carbohydrate accumulation in the fungus. Eventually, a stationary phase is reached. In some

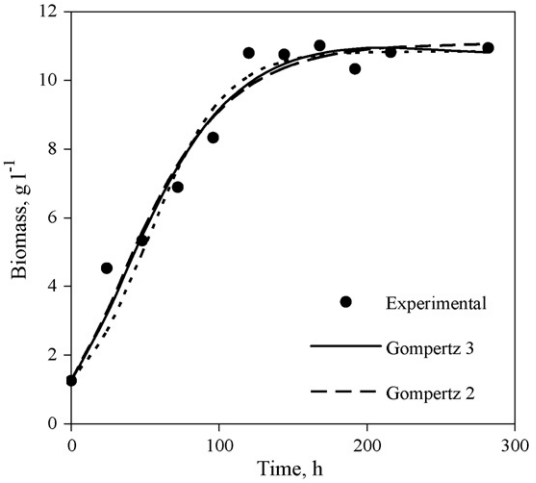

Fig. 1. Growth kinetic obtained during gibberellic acid production. Experimental and simulated data from the eleventh experiment (glucose, 50 g l^{-1}; NH_4Cl, 0.75 g l^{-1}; KH_2PO_4, 5 g l^{-1}; $MgSO_4{\cdot}7H_2O$, 1 g l^{-1}; trace elements, 2 ml l^{-1}; temperature, 29 °C; pH 3.0; aeration rate, 1.6 vvm; inoculum ratio, 10%).